Downregulation of LRRC8A-containing VRAC channels inhibits GBM proliferation and increases sensitivity to temozolomide and carmustine

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Introduction

• Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults.

• Ubiquitously expressed volume-regulated anion channels (VRAC) are believed to play a role in cell proliferation, migration, and apoptosis.

• VRAC are heteromeric channel complexes assembled with the mandatory LRRC8A subunit, which heteromerize with one or more complementary subunits (LRRC8B-E) from the leucine-rich repeat-containing 8 (LRRC8) family.

• By functionally inhibiting this channel, we aimed to decrease GBM cell proliferation.
Methods

• Primary GBM cells were derived from a surgical tissue sample. LRRC8A expression was determined with qRT-PCR and downregulated using gene-specific siRNAs.

• MTT proliferation assay was used to explore the effects of LRRC8A downregulation on proliferation and survival of GBM cells in the absence or presence of temozolomide, carmustine, or cisplatin. Chemotherapy dose-response experiments were first conducted to determine IC$_{50}$ values for this cell line.

• All data are expressed presented as the mean values ±S.E.M, after normalization to control values within the same experiment. Because of normalization, all comparisons to control values were performed using one-tailed t-test, followed by the Bonferroni post hoc correction for multiple comparisons.
Results

• Temozolomide, carmustine, and cisplatin reduced GBM cell proliferation and survival with the IC$_{50}$ values of ~1250, 320, and 30 µM, respectively. To reduce DMSO toxicity, we used temozolomide and carmustine at the concentrations below their IC$_{50}$ values.
Results

- Treatment with siLRRC8A_3 strongly reduced GBM proliferation by 55% (p<0.05) and significantly increased toxicity of 570 µM temozolomide by 34% (p<0.01).
Results

• Similarly, siLRRC8A_3 trended towards increased toxicity of 167 µM of carmustine by approximately 10% (p< 0.1).
Results

- In contrast, treatment with siLRRRC8A_3 nearly abolished sensitivity to 32 µM cisplatin, likely due to previously reported inhibition of cisplatin uptake via VRAC.
Discussion

• GBM proliferation, migration, and invasion have been found to be dependent on expression and activity of several types of $K^+$, $Ca^+$, non-selective cation, and $Cl^-$ channels.\(^1\)-\(^5\)

• Downregulation of the essential channel-forming subunit of VRAC, LRRC8A, appears to inhibit the rate of GBM cell proliferation by as much as 50% and can be used in combination with clinically approved treatment modalities.

• Future work must confirm these findings in multiple, diverse GBM cell lines and, subsequently, validate the effects of LRRC8A knockdown on GBM progression in animal models.


Summary Points

• To the best of our knowledge, this is the first study that has explored the functional significance of the LRRC8A-containing VRAC in human gliomas.

• Two major findings of our work include:

  1- Downregulation of LRRC8A strongly decreases proliferation of GBM cells.

  2- Downregulation of LRRC8A increases sensitivity of GBM cells to the clinically used chemotherapeutic agents temozolomide and carmustine, suggesting that VRAC may be a potential therapeutic target in the treatment of GBM.